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A Technique for Slide Preparation of Aphids, Scales, and Insect Genitalia and Larvae

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Describes step-by-step procedures for preparation of permanent slide mounts of several groups of insects, including aphids, scale insects, lepidoptera genitalia, and lepidoptera and sawfly larvae. Necessary equipment and reagents are listed.

Keyword: Insect collections.

Introduction

Many combinations of techniques, equipment, and reagents are suitable for mounting insects on slides (Clarke 1941, Essig 1948, Kozorzhevskaya 1968, and others). This Note does not attempt to review all of them, but outlines an efficient set of procedures for preparing slide mounts of four dissimilar groups, familiar to the author; undoubtedly other insects such as thrips could be added. Differences in handling the groups are minor, as outlined below.

Briefly, the procedure is (1) maceration (soaking) in potassium hydrohide (KOH) to soften body contents; (2) removal of body contents, embryos, scales, and so forth, in 50% alcohol; (3) staining in 50% alcohol into which several drops of a 0.5% solution of mercurochrome has been added; (4) cleanup and dehydration in 95% alcohol; (5) dehydration in 100% alcohol; (6) clearing in clove oil; (7) washing in xylene; (8) mounting in balsam; and (9) ringing. Five to twelve minutes per reagent is generally sufficient; exact times are best determined by experience.

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Equipment and Reagents

Necessary reagents are 10% solution of KOH; 50%, 95%, and 100% solutions of alcohol; 0.5% solution of mercurochrome; oil of cloves; xylene; mounting media; and a ringing compound. It is advisable to have a dropper bottle for each reagent except the mounting media. Use a standard balsam bottle for the mountant.

Equipment consists of a binocular microscope, hot plate, watch glasses, a spot plate (12 depression), small syringe, eye dropper, small camel's hair brush, slides, cover glasses and cover props, forceps whetted to a fine point, dissecting scissors with points whetted to a needle fineness, fine dissecting needles, miniature knives, and section lifters. Knives and section lifters can be obtained commercially or made by flattening, grinding, and polishing stainless steel insect pins or stainless steel wire. The wire may be obtained from salt-water fishing leaders or from piano wire. Surfaces that touch the specimens must be made as smooth as possible by honing and polishing with a fine abrasive. The tools are mounted in handles made of small-diameter wooden dowels. The type used by physicians as nasal applicators are especially suitable. Fine dissecting needles are made by forcing minutiens, insect pins, or fine sewing needles into handles made of toothpicks or small dowels.

Section lifters are used for transferring specimens from one solution to another, and for teasing out body contents, embryos, and so forth. The syringe is useful for transferring solutions when specimens are left in place. The miniature knives are used for cutting the genitalia from abdominal skins and splitting larval skins. A small camel's hair brush with all but a few bristles removed is useful for manipulating delicate specimens.

Procedures

Dry specimens are soaked in 100% alcohol before maceration in KOH (Post et al. 1968). When possible, select specimens in good condition with minimal damage to appendages. Fresh specimens are most suitable. Avoid using moths with abdomens glued on for genitalia mounts. Unless such a specimen is labeled indicating that the correct abdomen is attached, it should not be used (Clarke 1941).

Step 1.—Soften body contents

Use the same procedures for all insect groups. Heat at about 98°C in a 10% solution of KOH until body contents are soft, or if time permits, soak overnight at room temperature. To preserve natural colors, do not heat excessively; avoid boiling. For aphids and scales, some authors suggest punching a small hole through the integument in an inconspicuous place—generally somewhere on the abdomen—to facilitate the exchange of chemicals after the specimen is softened (Post et al. 1976, Kozorzhevskaya 1968).

Step 2.—Tease out body contents in 50% alcohol

It is not necessary to clean the specimens entirely at this stage; staining, dehydration, and clearing make body contents, scales, and other parts more visible and loosens them. The 50% solution of alcohol is sufficient to break the troublesome surface tension of the water.

Aphids and scales.—Tease out body contents and embryos carefully. If body scales are worked toward the rear, the anal area will usually rupture allowing body contents, embryos, etc. to escape. An alternative is to make a small cut on one side of the abdomen near the tip.

Genitalia.—Remove abdominal contents and loosen abdominal scales.

Larvae.—Cut off the head capsule and clean it out completely by teasing or by patting with a small section lifter. Later dehydration makes the head capsule brittle. Tease out body contents.

Step 3.—Staining

Use the same procedure for all groups. Place specimens in a small amount of a 50% solution of alcohol into which several drops of a 0.5% solution of mercurochrome have been added. Leave in the solution until specimens are pinkish to light red. Do not overstain.

Step 4.—Final cleanup and dehydration

Aphids.—Place in a 95% solution of alcohol and tease out any remaining body contents.

Genitalia.—Place in a 95% solution of alcohol. Clean off remaining scales and abdominal contents. If a female, cut off just ahead of the genital opening. If a male, cut off as close to the genitalia as possible. At this point, position the various parts of the genitalia for most advantageous viewing. This must be done before complete dehydration takes place. Both male and female abdominal skins are compressed laterally.

Larvae.—Place in 95% alcohol. Split the larval skin on one side just above the spiracles. Remove the remaining body contents and flatten the skin. One change is advisable. Small larval skins may be split by inserting a tiny knife inside the skin and rubbing a pin along the sharp edge. Larger skins are split with a fine dissecting scissors. To assure a good lateral view of a proleg, it is sometimes desirable to remove one and mount it separately. In the case of sawfly larvae, remove a mandible, an eyespot, and an antenna from the head capsule and cut away any portion which would be behind the antannae and tend to obscure it. It also may be helpful to pull off the mouthparts and mount separately.

Step 5.—Final dehydration

Aphids and genitalia.—Place in 100% alcohol and let remain for a few minutes. One change of alcohol is advisable.

Larvae.—Larval skin and head capsule are transferred to 100% alcohol for a few minutes.

Step 6.—Clearing

Use the same procedure for all groups. To improve brilliance, transfer the specimens to clove oil and let remain for a few minutes (Essig 1948).

Step 7.—Clearing and washing

Use the same procedure for all groups. Transfer specimens to xylene, and let remain for a few minutes.

Step 8.—Mounting on the slide

Use the same procedure for all groups. Clean a slide and cover glass with a dry cloth towel. Any tiny threads or lint from the towel may be easily blown off the slide or cover glass. Put a small amount of mounting media on the slide. Position the specimen and put the cover glass in place. When several tiny specimens are positioned under the same cover glass, they tend to flow with the mountant when the cover glass is added. To prevent this movement, place the specimens on a thin layer of mounting media, arrange them, and allow a film to form. Then add more mounting media and put the cover glass into place quickly. When cover glass props are used, place the cover glass on them before the mountant is added. If the cover glass props are thick, place a small drop of xylene under the cover glass and add the mountant with an eyedropper. Xylene speeds the flow of the mountant. The space under the cover glass must be filled quickly or the film will melt, allowing the specimens to flow with the mountant, possibly out from under the cover glass. Avoid the use of very thick balsam. Do not store completed mounts on edge when fresh.

Step 9.—Ringing

Use the same procedure for all groups. Ring with a suitable ringing compound to prevent darkening.

Discussion

Canada balsam is the most versatile mounting medium for small insects. Some of its most important qualities are that it gives up air bubbles and air pockets easily, rarely clouds or crystallizes, has a favorable refractive index, is cheap and readily available, and continues to clear after the specimen has been mounted. Some of the new synthetics seem to be suitable but have not been in use long enough to be proven.

Positioning the specimen in the mountant for most advantageous viewing is of the greatest importance. Characters necessary for determination must be in view, and their position from one slide to another must be uniform. If in doubt as to which characters are diagnostic, contact someone familiar with the group you are working on, ideally the taxonomist who will ultimately identify the specimens.

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